Attraction of Colorado Potato Beetle (Coleoptera: Chrysomelidae) to Damaged and Chemically Induced Potato Plants

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ABSTRACT Unmated adult female Colorado potato beetles, *Leptinotarsa decemlineata* (Say), were attracted to damaged and chemically treated potato plants in an olfactometer. Significantly more beetles moved upwind to potato plants with damage from larval Colorado potato beetle compared with undamaged plants. More beetles moved upwind toward potato plants treated with regurgitant from Colorado potato beetle larvae or cabbage looper larvae, *Trichoplusia ni* (Hübner), compared with undamaged plants. Greater numbers of beetles moved upwind toward potato plants treated with *N*-(17-hydroxylinolenoyl)-L-glutamine (volicitin) or to plants treated with the plant hormone methyl jasmonate, compared with untreated plants. Mechanical injury to potato foliage did not increase beetle attraction when plants were tested 1 or 24 h after injury. These results indicate that volatile chemicals produced by the plant in response to stimuli from feeding larvae enhance the attractiveness of the plant to female Colorado potato beetles.

KEY WORDS *Leptinotarsa decemlineata*, Colorado potato beetle, attraction, host-finding, volicitin

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is attracted to host plant odors and may use such odors both as a means of locating possible host plants and as a host selection mechanism. Adult *L. decemlineata* walk upwind in olfactometers in response to potato plant odors (McIndoo 1926, Schanz 1953, Jermy 1958). Upwind ambulation has also been demonstrated in a wind tunnel for both male and female beetles in response to potato plant volatiles (Visser 1976). Beetle responses to plant odor are somewhat specific, with positive anemotaxis occurring to a number of species of solanaceous plants related to potato, and not to most nonsolanaceous plants tested (Visser and Nielsen 1977). Such a response should lead beetles to plants in the family Solanaceae, after which other behavioral steps might proceed toward host selection and acceptance (Visser and Nielsen 1977).

The potato plant odorants that elicit anemotaxis (attraction) in Colorado potato beetle are unknown. A set of volatile compounds was isolated and identified from potato foliage by Visser et al. (1979), using vacuum steam distillation of homogenized foliage, followed by concentration of more volatile constituents in a cold trap and organic solvent extraction of the concentrate. Principal constituents of this extract were *trans*-2-hexenol, 1-hexenol, *cis*-3-hexen-1-ol, *trans*-2-hexenal, and linalool. The hexenols, *trans*-2-hexenal, and similar compounds are commonly encountered as volatiles from plant foliage and are referred to as green leaf volatiles (Visser and Ave 1978). Beetles did not respond significantly (did not move upwind) to any of these compounds, or to 2-hexenol or 3-hexenol, in a wind tunnel assay and inclusion of these chemicals with odor of potato foliage did not enhance beetle attraction to potato foliage (Visser and Ave 1978). The nature of host volatiles that are important elicitors of chemo-anemotaxis and important cues for host-finding behavior of Colorado potato beetle remains unknown.

Plant chemistry, including plant odor chemistry, may be greatly altered in response to herbivory (Turlings et al. 1995, Karban and Baldwin 1997). Herbivory, such as feeding by lepidopterous caterpillars, may result in immediate but short lived increases in release of plant odorants that are stored (constitutive) as well as the production and subsequent release of other chemicals over a longer time period as a response to herbivory (induced). For example, beet armyworm larvae, *Spodoptera exempta* (Walker), feeding on cotton plants cause the immediate release of 1 set of compounds (including the green leaf volatiles Z-3-hexenal and Z-3-hexenyl acetate, and the terpenes alpha pinene and caryophyllene) and stimulate the de novo biosynthesis and release of a different set of compounds, primarily acyclic terpenes, over a longer time period (Loughrin et al. 1994, Paré and Tumlinson 1997). Alborn et al. (1997) demonstrated the production and release of terpenoid compounds in corn with *N*-(17-hydroxylinolenoyl)-L-glutamine (volicitin), which was isolated and identified from beet armyworm regurgitant. The induced terpenoids are important host-finding chemicals for hymenopterous parasitoids of certain Lepidoptera larvae and it is suggested that these compounds may be released by

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plants to attract these parasitoids (Turlings et al. 1995).

Odor-mediated host-finding by herbivores may also be influenced by prior herbivory on host plants (Landolt 1993, Loughrin et al. 1998). Colorado potato beetles respond differently to damaged versus undamaged potato plants. Bolter et al. (1997) demonstrated increased attraction of Colorado potato beetles to potato plants that had been mechanically damaged or had been fed upon by conspecific larvae, and showed increases in quantities and numbers of volatiles released by such plants, compared with undamaged plants. The enhanced attractiveness of potato plants that were damaged mechanically or were injured from 30 min of larval feeding was short-lived. They noted greater longevity of the effects of injury (increased attractiveness to beetles) when potato plants were fed upon by larvae for 1 h or more, suggesting an increase in the production and release of plant volatiles that are attractive to Colorado potato beetles. However, potato plants that had been fed upon by *L. decemlineata* larvae became less attractive to adult beetles 24 h after the removal of those larvae. It is not clear if potato produces, de novo, chemicals that are attractive to Colorado potato beetle, in response to herbivory.

We conducted experiments to further evaluate the attractiveness of induced potato plants to female Colorado potato beetles. We demonstrated increased attractiveness of potato plants 24 h after damage by larval feeding, or after potato foliage was treated with regurgitant from Colorado potato beetle larvae or cabbage looper larvae, *Trichoplusia ni* (Hübner), after potato foliage was treated with volicitin, and after potato foliage was treated with methyl jasmonate, which appears to function as a plant hormone to stimulate the biosynthesis of defensive compounds in response to herbivory (Farmer and Ryan 1990; Bolter and Jongsma 1995). These findings indicate an important role of potato plant odorants that are induced by herbivory in Colorado potato beetle host finding behavior.

Materials and Methods

General. Colorado potato beetles were obtained in August/September of 1997 as larvae on Russet-Burbank potato near Moxee, Yakima County, WA. Mature larvae were placed in plastic canisters (4 liter) with cut potato foliage over sand. After cessation of feeding, larvae pupated in the sand. Pupae in sand were held in a controlled environment room for adult emergence (22°C, 60% RH, and a photoperiod of 16:8 [L:D] h). Adults were separated by sex on the 1st d after emergence. Subsequent generations were reared using the same procedure, with eggs obtained from mated females placed in plastic boxes (16 by 33 by 9 cm) with potato foliage and with larvae reared on potted potato plants. The beetle colony was maintained in a controlled environment chamber at 22–23°C and a photoperiod of 16:8 (L:D) h. Newly emerged beetles were sorted by sex when 1–2 d old. They were kept in plastic boxes with screened lids in a controlled environment room and were provided cut potato foliage daily unless otherwise indicated. Beetles used in bioassays were 6–14 d old and were held without potato foliage or other food for 24 h before testing. All assays were conducted in the 4th through the 8th h of the photophase.

Potato plants used for beetle rearing and in these experiments were Russet-Burbank, obtained as seed potatoes that were held in long-term cold storage (2°C). Potato eyes were removed and were planted individually 2.5 cm deep in 15-cm-diameter plastic pots of soil. Soil was a 3:1 mix of sand and peat. Pots were held in a greenhouse under both sodium and metal halide lamps as supplemental lighting. Lamps were on a photoperiod of 12:12 (L:D) h and plants for these experiments were grown from November 1997 through May 1998. Single potato plants used in bioassays were removed from pots 24 h before experiments by grasping the plant at the base of the stem and washing the soil off the roots with water. The plant was then placed in a 100-ml glass beaker with the roots immersed in 80 ml of water. Plants in beakers remained in the greenhouse until 1 h before experiments, when they were transported to a controlled environment room housing the olfactometers. All plants tested were 4–6 wk of age and were 12–15 cm in height. Pairs of plants for assays were selected for identical age and for similarity of height and shape. Unless otherwise stated, plants used in experiments were free of insects and insect damage.

Bioassays. A straight tube olfactometer design was used. The olfactometer consisted of 3 parts that were connected in series; an air filter, then the odor chamber, and an assay chamber. Room air was pushed through the system with a small pump. This air was provided via a flow meter at 75 ml/min through the olfactometer. For the purification of pumped air, airflow was passed through a charcoal filter. The odor chamber was used as a means of introducing treatment odors into the olfactometer. The odor chamber was a 4-liter glass jar fitted with a Teflon lined lid fitted with brass inlet and outlet ports (0.635 cm i.d.) for air exchange. Airflow, provided by the pump and flowmeter and purified by the charcoal filter, passed through this odor chamber and was then vented via stainless steel tubing into the assay chamber. The assay chamber was a glass tube (37 cm long by 2.5 cm i.d.) through which the airflow exiting the odor chamber was passed. This tube was modified for the attachment underneath of an 125-ml Erlenmeyer flask 5 cm from the upwind end of the tube, which functioned as a trap to collect beetles that moved >32 cm upwind. Two systems were used in parallel for comparison of a treatment and a control. At the beginning of each assay, a 3rd flow meter was used to check the volume of vented air for each olfactometer, to be sure they were comparable. Plants set in 100-ml glass beakers filled with water were placed within odor chambers 1 h before assays were begun and airflow was passed through each system at 300 ml/min for 1 h to purge the system. Between assays, all glassware was washed with hot soapy water, rinsed with acetone, rinsed with hexane, and baked in a convection oven at 140°C for 24 h. The standard protocol for each assay consisted of placing 5 beetles at the downwind end of each assay chamber 1 h after the olfactometers were set up, and counting the numbers of trapped beetles (beetles that moved upwind and fell into the flask at the upwind end of the assay chamber) after 30 min. After 1 assay was conducted in this manner, the positions of the olfactometers were switched (to control for position effects) and another 5 beetles were tested in each of the 2 assay chambers. All hardware was then cleaned as indicated above before it was used in another assay.

Experimental Descriptions. Eight experiments were conducted using the straight tube olfactometer system described. These experiments compared rates of responses of mature but unmated female beetles to potato plants treated in various ways to cause or simulate herbivory, in comparison to appropriate controls. Generally, assays involved the testing of 5 beetles per plant treatment, with the assay replicated 10 times, providing 50 beetles tested per plant treatment. Additionally, an experiment was conducted to evaluate males, unmated females, and mated females in their responses to induced potato plants.

Response to Undamaged Potato Plant. A comparison was made of beetle attraction to a single uninjured potato plant and the system control (metered, purified air with no plant in the odor chamber) to determine if beetles were attracted to potato plants in the olfactometer system. The treatment was a potato plant placed in the olfactometer odor chamber (4-liter glass jar) 1 h before the start of assays. A glass beaker containing 80 ml of water was placed in the odor chamber 1 h before assays, as the control.

Response to Hour Old Mechanical Damage. A comparison was made of beetle attraction to a potato plant with fresh mechanical damage compared with a potato plant that was untreated and uninjured. To produce mechanical damage, 3 leaves near the top of a potato plant were cut with scissors (5 cm cuts), with 1 cut per leaf along the long axis of the leaf. Plants were moved to jars of water 24 h before assays and cuts to leaves were made immediately before plants were placed in the odor chambers of the olfactometer, 1 h before the onset of bioassays. These plants were compared with identical but uncut plants.

Response to Day-Old Mechanical Damage. A comparison was made of beetle attraction to a potato plant with day-old mechanical damage compared with a potato plant that was untreated and uninjured. Three top leaves of a potato plant were cut with scissors (5 cm cuts) along the long axis of the leaf to produce the mechanical damage. Plants were cut in the greenhouse immediately before they were moved from pots of soil to beakers of water, 24 h before assays. They remained in the greenhouse until 1 h before assays, when they were placed in the odor chambers. These were compared with identical but uncut plants.

Response to Colorado Potato Beetle-Infested Potato. A comparison was made of beetle attraction to a potato plant on which Colorado potato beetle larvae had fed and a potato plant that was untreated and uninjured. Five 4th instars were placed on plants in the greenhouse 24 h before assays, when plants were moved from pots of soil to beakers of water. Beetle larvae were then removed 1 h before assay initiation when plants were placed in the odor chambers of the olfactometer. These were compared with comparable but undamaged plants free of larvae.

Response to Colorado Potato Beetle Regurgitant-Treated Potato. A comparison was made of beetle attraction to a potato plant treated with regurgitant from 4th-instar Colorado potato beetle larvae and to a potato plant that was untreated. Applications of regurgitant were made in the greenhouse 24 h before assays, when plants were moved from pots of soil to beakers of water. A razor blade was used to scrape an upper leaf surface (≈ 1 cm^2) and a beetle larva was gently squeezed until a droplet of regurgitant was produced at the mouth. This was then applied to the scraped area of the leaf. A different larva was used for each scrape, with 3 scrapes and regurgitant applications made per plant. Plants were placed in the odor chambers of the olfactometer 1 h before assays were started. These plants were compared with comparable scraped plants that were not treated with regurgitant. Eighty beetles were tested for responses to each treatment, as 16 groups of 5 beetles per plant treatment.

Response to Cabbage Looper Regurgitant-Treated Potato. A comparison was made of beetle attraction to potato plants treated with regurgitant from 5th-instar cabbage looper larvae and to potato plants that were untreated. Late instar cabbage looper larvae were acquired from a laboratory insectary and were fed a pinto bean-based artificial diet. Cabbage looper regurgitant was applied to scraped areas of potato leaves as described above for potato plant treatment with Colorado potato beetle regurgitant. Regurgitant from a cabbage looper larva was applied to the scraped area of the leaf. A different larva was used for each scrape, with 3 scrapes and regurgitant applications made per plant. This plant was then moved into the odor chamber of the olfactometer 1 h before assays were started. These were compared with comparable plants that were scraped but were not treated with cabbage looper regurgitant.

Response to Volicitin-Treated Plants. A comparison was made of beetle attraction to potato plants treated with volicitin and to plants that were untreated. Synthetic volicitin (Alborn et al. 1997) was applied to a razor scrape to 1 leaf at a 15 microliter dose, with 1 application made per plant. Volicitin concentration was 40 nanograms per microliter in 50 micromolar phosphate buffer. Application of volicitin was made in the greenhouse 24 h before assays, when plants were moved from pots of soil to beakers of water. These plants were compared with comparable plants that were similarly scraped with a razor blade 24 h before assays but were not treated with volicitin.

Response to Methyl Jasmonate-Treated Potato. A comparison was made of beetle attraction to potato plants treated with methyl jasmonate and to undamaged and untreated potato plants. Treated potato plants were placed in 80 ml of an aqueous solution of

Table 1. Percentages of Colorado potato beetles (CPB) attracted in a straight tube olfactometer to a series of pairs of control versus treated plants

Treatment	Mean ± SE	*t*	*P*	*n*
System control	20 ± 5.9			10
Undamaged potato plant	36 ± 5.0	3.2	0.01	10
Undamaged potato plant	36 ± 7.2			10
1-h mechanical damage	46 ± 9.0	1.24	0.24	10
Undamaged potato plant	38 ± 8.1			10
24-h mechanical damage	40 ± 6.0	0.29	0.78	10
Undamaged potatio plant	30 ± 5.4			10
CPB damaged potato plant	66 ± 8.5	4.32	0.002	10
Undamaged potato plant	31 ± 3.6			16
CPB treated potato plant	51 ± 4.8	3.65	0.002	16
Undamaged potato plant	32 ± 6.1			10
CL treated potato plant	58 ± 5.5	2.75	0.02	10
Undamaged potato plant	36 ± 7.2			10
Volicitin-treated potato plant	62 ± 5.5	3.28	0.01	10
Undamaged potato plant	43 ± 4.0			22
Methyl jasmonate treated plant	61 ± 4.5	3.36	0.003	22

methyl jasmonate (2 μg/ml of water) 24 h before bioassays in the greenhouse when plants were moved from pots of soil to beakers of water. Control plants were placed in water with no methyl jasmonate. Ten assays were conducted, providing 100 beetles tested to the treatment and 100 beetles were tested to the control. One hundred and ten beetles were tested for responses to each plant treatment, as 22 groups of 5 beetles tested to the untreated plants and 22 groups of 5 beetles tested to the methyl jasmonate treated plants.

Male and Female Responses to Colorado Potato Beetle Regurgitant Treated Potato. One of the above experiments, testing for effects of Colorado potato beetle regurgitant on the attractiveness of potato plants, was repeated to determine if responses of males, unmated females, and mated females of the Colorado potato beetle to regurgitant-treated plants are enhanced in comparison to untreated plants. Eighty male, 160 unmated female, and 105 mated female beetles were tested for attraction to untreated plants and like numbers of each category were tested for attraction to potato plants treated with regurgitant from larval Colorado potato beetle. Treatment and bioassay methods were as described above. Beetles were assayed as groups of 5, providing 16 assay replicates for males, 32 assay replicates for unmated females and 26 assay replicates for mated females.

For each experiment, mean response data for untreated or control plants were compared with the mean response data for treated plants by a paired *t*-test.

Results

Female Colorado potato beetles were attracted to odor from potato foliage. Significantly greater numbers of unmated female Colorado potato beetles moved upwind and were trapped in straight tubes carrying airflow from over a potato plant compared with the system control (Table 1).

Beetles did not respond better to mechanically damaged potato foliage. Numbers of beetles that moved upwind toward potato plants damaged by scissor cuts 1 h before assays were greater but were not significantly higher than numbers that moved upwind toward undamaged potato plants (Table 1). Similarly, numbers of beetles that moved upwind toward potato plants damaged by scissor cuts 24 h before assays were not significantly higher than numbers that moved upwind toward undamaged potato plants (Table 1).

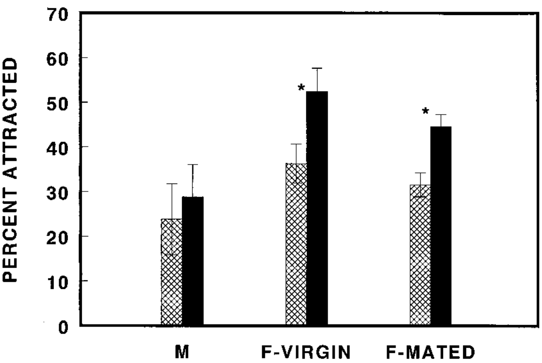

Fig. 1. Mean ± SE percentages of male, unmated female, and mated female Colorado potato beetles in an olfactometer that moved upwind toward undamaged Russet Burbank potato plants (crosshatched bars) or toward potato plants treated 24 h earlier with regurgitant from larvae of the Colorado potato beetle (solid bars). An asterisk above a pair of bars indicates they are significantly different by a paired *t*-test at $P \leq 0.05$.

Beetles did respond significantly better to potato plants fed upon by Colorado potato beetle larvae or treated with insect regurgitant. Significantly greater numbers of beetles moved upwind toward potato plants that were fed upon by Colorado potato beetle larvae for the 23 h before experiments compared with numbers attracted to undamaged plants (Table 1). Greater numbers of beetles moved upwind toward potato plants treated with regurgitant from Colorado potato beetle larvae compared with untreated plants (Table 1). Similarly, significantly greater numbers of beetles moved upwind to potato plants treated with regurgitant from larval cabbage loopers, compared with untreated plants (Table 1).

Chemical treatments that were expected to induce potato plant biosynthesis of odorants also increased their attractiveness to female Colorado potato beetles. Significantly greater numbers of beetles moved upwind toward potato plants treated with volicitin applied to leaves compared with untreated potato plants (Table 1). Potato plants in water containing methyl jasmonate elicited attraction responses from greater numbers of beetles than potato plants placed in water without methyl jasmonate (Table 1).

Male Colorado potato beetles did not respond better to potato plants treated with conspecific larval regurgitant compared with untreated and uninjured plants (Fig. 1). This is in contrast to the results with both mated and unmated females, which responded significantly better to regurgitant treated plants than untreated plants (Fig. 1) with greater numbers moving upwind toward treated plants.

Discussion

Our results repeatedly demonstrated enhanced attraction of virgin female Colorado potato beetles to potato plants that were treated to induce the production of defensive compounds, including odorants. The feeding by larvae on plants, application of regurgitant from Colorado potato beetle larvae or cabbage looper larvae, application of volicitin, and treatment with methyl jasmonate, have all been implicated in the stimulation of induction of defensive chemistry in plants. Feeding by Colorado potato beetle larvae on potato plants (Bolter et al. 1997) or beet armyworm larvae on cotton plants results in both the short-term release of constitutive odorants and the stimulation of production and release of induced odorants (Loughrin et al. 1994; Turlings et al. 1995). The application of regurgitant from beet armyworm larvae to foliage is known to induce the production of odorants from corn and cotton plants (Turlings et al. 1990, Loughrin et al. 1994). Methyl jasmonate and jasmonic acid have been implicated in the process of induction of defensive chemistry within the plant (Farmer and Ryan 1990, Bolter and Jongsma 1995). Volicitin, isolated and identified from beet armyworm larval regurgitant, induces corn seedlings to emit volatiles that are characteristic of larval damaged plants (Alborn et al. 1997). This series of experiments provides strong evidence that Colorado potato beetles are attracted to odors of potato plants that are attacked by chewing insects and are induced to produce defensive chemicals, including odorants, in response to specific stimuli from the attacking organisms.

Our results with plants that were damaged mechanically are consistent with previous findings. We found no effect of cuts from scissors on plant attractiveness, neither 1 nor 24 h after treatment. Bolter et al. (1997) found that similar injuries to foliage resulted in both increases in release of odorants and increased attractiveness of plants to beetles, but for a very short period of time (15 min). Apparently, the constitutive odorants of potato that are attractive to Colorado potato beetle decrease rapidly over time after injury or cessation of injury.

Laboratory experiments indicate that potato plants infested with Colorado potato beetle larvae are more attractive to Colorado potato beetle adult females than are uninfested plants (Bolter et al. 1997, and herein). The possible sources of this increased attractiveness are, however, multiple. Plants with actively feeding larvae probably release constitutive volatiles as a result of continuous new injury to plant foliage (Bolter et al. 1997). Feeding by Colorado potato beetle larvae probably also stimulates the induction of de novo biosynthesis of volatiles by the potato plant, as occurs in other plant systems (i.e., Paré and Tumlinson 1997). The release of additional chemical odorants from the larvae themselves and from larval frass also cannot be ruled out, and microbial volatiles may be produced from leaf surfaces contaminated with frass. The increased attractiveness of plants harboring beetle larvae could then originate from several odorant sources, and could be complicated by the simultaneous presence of attractants and repellents or deterrents. However, our experiments treating plants with beetle and caterpillar regurgitant, volicitin, or methyl jasmonate, demonstrated that induced odorants are responsible, in part, for the increased attractiveness of infested plants.

It is possible to treat crops in the field with chemicals that will stimulate the de novo biosynthesis of defensive compounds in plants, thereby providing protection against microbial and insect attack. Agrawal (1998) demonstrated beneficial effects of herbivore damage to radish plants, including lowered rates of attack by other chewing herbivores and by aphids. The assumption can be made that this induced response by the plant is beneficial to the plant and is effective generally against attacking insects and diseases. However, as pointed out by Karban and Baldwin (1997), specialist herbivores may respond positively to such induced chemicals as appropriate host cues, whereas generalist herbivores may be negatively affected. This opinion is supported here, where the Colorado potato beetle, a specialist on a small number of species of related plants, responds positively to potato plants that have been damaged by larvae, treated with insect regurgitant, volicitin, or methyl jasmonate, to induce the production of odorants. It may be necessary to investigate individually the responses of targeted insect pests on different crops to these plant chemical changes before proceeding with such treatments as crop protectants.

Colorado potato beetles may respond more vigorously to plants that are damaged or injured in a variety of ways. Beetle attraction is enhanced by exposure of potato plants to damaging levels of ozone (Schutz et al. 1995), to mechanical damage (Bolter et al. 1997) and to feeding by beetle larvae on plant foliage (Bolter et al. 1997). It remains to be determined if this enhancement of attraction is a result of increased amounts of plant odorants released (Bolter et al. 1997), qualitative changes in plant odorants emitted by potato foliage, or both. It was suggested by Bolter et al. (1997) that the Colorado potato beetle responds both to plant volatiles that are constitutive and plant volatiles that are induced, and that induced volatile compounds of potato are the same as those released constitutively. Further work is required to determine which odorants released by damaged or treated potato plants elicit anemotactic responses in Colorado potato beetle.

It is still not understood how Colorado potato beetles may use host odors to find host plants in the field. This study and previous investigations using laboratory ambulatory assays have implicated host plant volatiles as attractants for this beetle, but field verification of such behavior is lacking. Also, Colorado potato beetle flight responses to plant odors have not been demonstrated, and this would be important to host-finding over any significant distance. With positive effects of both constitutive and induced odorants on beetle attraction to potato plants, it is not yet known if compounds from one or the other or both classes might prove to be of primary importance in beetle host-finding. Previous attempts to identify host odorants that are attractive to beetles have been disappointing, with no clear evidence of beetle orientation in response to particular plant volatiles (Visser et al. 1979). Perhaps, with a better understanding of the effects of plant injury on odor chemistry and how it influences Colorado potato beetle behavior, progress can be made in duplicating an aroma that the female beetle finds enticing.

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